

# UC Davis

## UC Davis Previously Published Works

### Title

Linoleic Acid-Rich Oil Supplementation Increases Total and High-Molecular-Weight Adiponectin and Alters Plasma Oxylipins in Postmenopausal Women with Metabolic Syndrome

### Permalink

<https://escholarship.org/uc/item/8zc8j31w>

### Journal

Current Developments in Nutrition, 4(9)

### ISSN

2475-2991

### Authors

Cole, Rachel M  
Puchala, Sarah  
Ke, Jia-Yu  
[et al.](#)

### Publication Date

2020-09-01

### DOI

10.1093/cdn/nzaa136

Peer reviewed



# Linoleic Acid–Rich Oil Supplementation Increases Total and High-Molecular-Weight Adiponectin and Alters Plasma Oxylipins in Postmenopausal Women with Metabolic Syndrome

Rachel M Cole,<sup>1</sup> Sarah Puchala,<sup>1</sup> Jia-Yu Ke,<sup>1</sup> Mahmoud Abdel-Rasoul,<sup>2</sup> Kristin Harlow,<sup>3</sup> Benjamin O'Donnell,<sup>4</sup> David Bradley,<sup>4</sup> Rebecca Andridge,<sup>3</sup> Kamil Borkowski,<sup>5</sup> John W Newman,<sup>5,6,7</sup> and Martha A Belury<sup>1</sup>

<sup>1</sup>Program of Human Nutrition, Department of Human Sciences, The Ohio State University, Columbus, OH, USA; <sup>2</sup>Center of Biostatistics, The Ohio State University, Columbus, OH, USA; <sup>3</sup>College of Public Health, The Ohio State University, Columbus, OH, USA; <sup>4</sup>Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA; <sup>5</sup>West Coast Metabolomics Center, Genome Center, University of California Davis, Davis, CA, USA; <sup>6</sup>USDA Agricultural Research Service Western Human Nutrition Research Center, Davis, CA, USA; and <sup>7</sup>Department of Nutrition, University of California Davis, Davis, CA, USA

## ABSTRACT

**Background:** The onset of menopause increases the risk of metabolic syndrome (MetS). Adiponectin is an adipokine associated with insulin sensitivity that is lower in people with MetS. Supplementing diets with linoleic acid (LA)-rich oil increased adiponectin concentrations and improved glucose control in women with type 2 diabetes. The effect of LA on adipokines, especially total and the bioactive form of adiponectin, high-molecular-weight (HMW) adiponectin, in women with MetS is unknown.

**Objectives:** The aim of this study was to explore the effect of supplementation of the diet with an oil rich in LA on adipokines in women with MetS. The effect of the LA-rich oil (LA-oil) on oxylipins, key metabolites that may influence inflammation and metabolism, was also explored.

**Methods:** In this open-label single-arm pilot study, 18 postmenopausal nondiabetic women with MetS enrolled in a 2-phase study were instructed to consume LA-rich vegetable oil (10 mL/d) as part of their habitual diets. Women consumed an oleic acid–rich oil (OA-oil) for 4 wk followed by an LA-oil for 16 wk. Fasting concentrations of adipokines, fatty acids, oxylipins, and markers of glycemia and inflammation were measured.

**Results:** After 4 wk of OA-oil consumption, fasting glucose and total adiponectin concentrations decreased whereas fasting C-reactive protein increased. After 16 wk of LA-oil supplementation total and HMW adiponectin and plasma oxylipins increased. Markers of inflammation and glycemia were unchanged after LA-oil consumption.

**Conclusions:** Supplementation with LA-oil increased total and HMW adiponectin concentrations and altered plasma oxylipin profiles. Larger studies are needed to elucidate the links between these changes and MetS. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02063165. *Curr Dev Nutr* 2020;4:nzaa136.

**Keywords:** linoleic acid, metabolic syndrome, adiponectin, oxylipin, fatty acid composition, adherence

Copyright © The Author(s) on behalf of the American Society for Nutrition 2020. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

Manuscript received June 4, 2020. Initial review completed July 28, 2020. Revision accepted August 4, 2020. Published online August 21, 2020.

Supported by the Ohio Agricultural Research and Development Center (Wooster, Ohio) (to MAB), the Carol S Kennedy Professorship (to MAB), and NIH grant UL1TR001070 from the National Center for Advancing Translational Sciences. Additional support was provided by USDA Intramural Project 2032-51530-022-00D (to JWN) and NIH grant U24 DK097154 (to JWN).

Author disclosures: All authors report no conflicts of interest.

The USDA is an equal opportunity employer and provider. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Advancing Translational Sciences, the NIH, or other sponsors of this work.

Supplemental Tables 1–3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/cdn/>.

Address correspondence to MAB (e-mail: [belury.1@osu.edu](mailto:belury.1@osu.edu)).

Abbreviations used: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; ALM, appendicular lean mass; CRP, C-reactive protein; CUDA, 1-cyclohexyl ureido, 3-dodecanoic acid; DEA, docosahexaenylethanolamide; DiHDOPA, dihydroxydocosapentaenoic acid; DiHOME, dihydroxyoctadecenoic acid; EpO, epoxyoctadecanoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; HMW, high molecular weight; HODE, hydroxyoctadecadienoic acid; KODE, keto-octadecadienoic acid; LA, linoleic acid; LA-oil, linoleic acid-rich oil; LMM, lean muscle mass; MetS, metabolic syndrome; OA, oleic acid; OA-oil, oleic acid–rich oil; oxLDL, oxidized low-density lipoprotein; PPAR $\gamma$ , peroxisome proliferator–activated receptor  $\gamma$ ; PUHA, 1-phenyl ureido 3-hexanoic acid; SPE, solid phase extraction; TNF-R2, TNF-receptor 2.

## Introduction

Metabolic syndrome (MetS) is a group of related conditions including abdominal obesity; elevated triglycerides, glucose, and blood pressure; and low HDL cholesterol (1–3). MetS is associated with higher

markers of inflammation and oxidative stress (4) and increases the risk of developing heart disease (5–7) and diabetes (7, 8). The prevalence of MetS increases with age (9–11) and postmenopausal status (11, 12). Indeed, postmenopausal women often experience an increase in adipose mass (13–15), with a shift in adipose to the abdominal

region (13, 15), and a decrease in lean mass (13, 14). Furthermore, compared with premenopausal women, postmenopausal women have higher fasting insulin, fasting glucose, triglycerides, and blood pressure (16).

MetS is associated with reduced concentrations of both total adiponectin (4, 17) and high-molecular-weight (HMW) adiponectin (18). Adiponectin is a peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) responsive gene product (19) secreted primarily from adipose tissue (20). The HMW form of adiponectin may be the more biologically active form (21). Adiponectin concentrations are negatively associated with insulin resistance (22), waist circumference, and adipose mass (23), and positively associated with lean mass (24) in postmenopausal women. Although adiponectin concentrations often increase in women after menopause (22), postmenopausal women with MetS have reduced concentrations of adiponectin when compared with postmenopausal women without MetS (25), suggesting that adiponectin may be a potential target for the treatment of MetS especially in postmenopausal women.

Linoleic acid (LA) is an essential dietary PUFA that is decreasing in many US crop seed oils (26, 27). Yet, increasing LA in the diet, even without changing other aspects of the diet or dietary fat intake, positively alters adiponectin concentrations, insulin sensitivity (28, 29), and body composition (28, 30, 31). Furthermore, higher blood concentrations of LA are associated with reduced risk of type 2 diabetes (32). LA can be oxygenated either enzymatically or through interactions with reactive oxygen to form oxylipins (33, 34), lipid mediators that are altered in plasma lipoprotein of individuals with MetS (35). In addition, LA (36) and some LA-oxylipins (37–39) are believed to be PPAR $\gamma$  agonists and thus have the potential to influence adiponectin concentrations.

The objectives of this pilot study were to determine the impacts of LA-oil supplementation on total and HMW adiponectin and explore the impact of LA-oil supplementation on oxylipin profiles in postmenopausal women with MetS.

## Methods

### Study design and participants

This pilot and feasibility study (NCT02063165) was a single-arm, 2-phase, open-label intervention study approved by the Ohio State University Institutional Biomedical Review Board (protocol number 2012H0259). All participants provided written informed consent before study participation. This study was conducted according to the Declaration of Helsinki. Partial descriptions of the study design, methods, participant inclusion and exclusion criteria, and participant characteristics have been previously reported (24).

Overweight or obese postmenopausal women were recruited from the greater Columbus Ohio area over a 1-y period (2014–2015). Inclusion and exclusion criteria were verified at prescreening and during the screening visit. Inclusion criteria were female, postmenopausal ( $\geq 1$  y without menses), age  $\geq 50$  and  $\leq 69$  y, BMI (in kg/m<sup>2</sup>)  $\geq 25$  and  $\leq 55$ , waist circumference  $\geq 88$  cm, stable medical therapy and weight for  $\geq 3$  mo, and  $\geq 1$  of the following: triglyceride  $> 150$  mg/dL or triglyceride-lowering medication, HDL cholesterol  $< 50$  mg/dL or lipid-lowering medication, blood pressure  $> 130$  mm Hg systolic or  $> 85$  mm Hg diastolic or blood pressure-lowering medication, and blood glucose  $> 100$

and  $< 126$  mg/dL. Exclusion criteria included self-reported substance abuse; impaired cognitive function; current or previous diagnosis of heart, renal, or circulatory diseases or type 2 diabetes; current use of supplements or medications known to affect body composition; current or previous use of oral hypoglycemic agents or exogenous insulin; and gastrointestinal diseases or disorders.

Study visits began between 06:00 and 09:30 after an overnight fast for  $\geq 10$  h and abstaining from consuming the study oils on the morning of each study visit. Participants were asked not to alter their dietary intake or physical activity throughout the study. Participants received the placebo oil [oleic acid (OA)-rich oil; OA-oil] during a first 4-wk run-in period (weeks  $-4, 0$ ) and then received the LA-oil for the 16-wk dietary intervention period (weeks 0, 4, 8, 12, and 16). Participants were asked to return unused oils and report any adverse events at each study visit.

### Dietary oils

Participants were instructed to consume 1 teaspoon (5 mL) of the study oil twice per day during the entire study. Participants were given examples of ways to incorporate the oils into their diet without using the oils for cooking or baking. Participants consumed high-OA safflower oil (LouAna Oils) during the run-in period (7.3 g OA per 9.3 g fat) and high-LA safflower oil (Arista Industries, Inc.) during the intervention period (6.9 g LA per 9.3 g fat). Fatty acid composition of the oils was verified every 3 mo. The OA-oil for the run-in has a similar fatty acid composition to the LA-oil used during the intervention period, with the substitution of OA for LA (**Supplemental Table 1**). Owing to inability to acquire LA-rich safflower oil toward the end of the study, 2 participants were given grapeseed oil, 1 from week 12 to week 16 and 1 from week 8 to week 16, during the intervention. The grapeseed oil (Pompeian Inc.) contained the same amount of LA (6.9 g LA per 9.3 g fat) and an almost identical fatty acid composition to the high-LA safflower oil.

### Adherence to protocol

To assess adherence, participants received 1 bottle of preweighed oil at each study visit and a 1-teaspoon measuring spoon at the beginning of the study to measure the oils. Each bottle of returned oil was weighed to calculate the oil consumed during each 4-wk interval between study visits. In addition, participants were instructed to self-record daily consumption. Expected oil consumption each day was 9.3 g or 10 mL (2 teaspoons) during both the run-in and intervention periods.

### Fatty acids

Fasting blood samples collected at each study visit were prepared for plasma and erythrocyte samples and used for fatty acid analysis. A subset of 12 women consented to receive adipose biopsies performed by a medical doctor at weeks 0 and 16 as previously described (40). Total lipids were extracted from plasma and adipose samples using chloroform:methanol (2:1, vol:vol) and 0.88% potassium chloride wash (41). FAMES from plasma, adipose, and study oils were prepared using 5% hydrochloric acid in methanol (42) at 76°C. Erythrocyte fatty acids were extracted and methylated using boron trifluoride at 100°C (43–45). Analysis of all FAMES was completed as previously described (46). Retention times of sample FAMES were compared to standards (Matreya, LLC, and Nu-Check Prep Inc.). The percentage of each fatty acid from the total identified is reported (47).

### Oxylipins and other lipid mediators

Lipids were first isolated using 10:8:11 (vol:vol:vol) cyclohexane:isopropanol:ammonium acetate and treated with a mineral base to release free fatty acids from esterified lipid pools. Alkaline stable products were then purified by solid-phase extraction and quantified by electrospray ionization LC–tandem MS as previously described (48, 49). Briefly, 100  $\mu$ L of plasma was enriched with butylated hydroxytoluene, EDTA, and deuterated surrogates in methanol, diluted with isopropanol, homogenized on a mechanical bead beater, and extracted with cyclohexane. The extract was split into organic and aqueous phases with 0.1 M ammonium acetate and centrifugation at 4°C for 5 min at 10,000  $\times$  *g*. The aqueous phase was re-extracted with cyclohexane, then the organic phases were combined, dried under vacuum, and reconstituted in 40  $\mu$ L 1:1 methanol:toluene (vol:vol). To release esterified lipids, the 40  $\mu$ L total lipid extract was mixed with 100  $\mu$ L 0.5 M methanolic sodium methoxide, topped with nitrogen, mixed, and incubated for 1 h at 60°C. A 100- $\mu$ L aliquot of water was then added, the vial was flushed with nitrogen, and then was returned to 60°C for another hour. This 2-step procedure promotes efficient transformation of lipophilic esters including cholesterol esters and triglycerides. Oxylipin free acids were then isolated from cooled samples with 1-cc 10-mg Oasis HLB solid-phase extraction (SPE) 96-well plates (Waters Corp Inc.). The SPE wells were washed with ethyl acetate and methanol and conditioned with 2 mL 0.1% arachidonic acid (AA; 20:4n–6) / 5% MeOH. Hydrolysates were diluted with 0.5 mL 0.1% AA / 5% MeOH and neutralized with 10  $\mu$ L 20% glacial acetic acid (~0.17 M), transferred to the SPE wells, and further diluted with 0.5 mL wash solution, which was used to rinse the hydrolysate vial to yield a final solution with 16% organics. The sample was loaded onto the column and extracted by gravity, air dried for 20 min with a light vacuum, and mediators were eluted with 0.5 mL 1.0% acetic acid in methanol, followed by 1.0 mL ethyl acetate, and collected in deep-well polypropylene 96-well plates containing 10  $\mu$ L 20% glycerol in methanol. Solvents were removed under a vacuum, then residues were reconstituted in 100  $\mu$ L 100 nM cyclohexylureidododecanoic acid (Sigma-Aldrich) internal standard solution, chilled, and filtered by centrifugation for ~1 min through a 0.2- $\mu$ m polyvinylidene difluoride membrane 400- $\mu$ L 96-well microfilter plate (Agilent Technologies), with eluents collected in 0.45-mL polypropylene microplates. Filtered samples were mat-capped and analyzed directly against authentic calibration standards on an API 6500 QTRAP (Sciex) after residue separation on a 2.1  $\times$  150 mm 0.17- $\mu$ m BEH column (Waters Corp). Analytes were generally quantified against 6- to 9-point calibration curves. Signals for the parent fatty acids, acylethanolamide of EPA (20:5n–3) and palmitoleic acid (16:1n–7), were assessed based on published mass transitions and retention times relative to quantified compounds, and are reported here as relative abundance across the experimental data sets. Calibrants and internal standards were either synthesized [(1-cyclohexyl ureido, 3-dodecanoic acid (CUDA), 1-phenyl ureido 3-hexanoic acid (PUHA)] or purchased from Cayman Chemical, Medical Isotopes, Avanti Polar Lipids Inc., or Larodan Fine Lipids. Data were processed with AB Sciex MultiQuant version 3.0.

### Biochemical analysis

Fasting concentrations of triglyceride, HDL cholesterol, LDL cholesterol, and total cholesterol were measured from whole blood obtained

from a finger stick (Cholestech LDX analyzer, Cholestech Corporation). Fasting serum glucose, insulin, and TNF-receptor 2 (TNF-R2) concentrations were analyzed by the Clinical Research Core laboratories for all study visits (at the Ohio State Wexner Medical Center). Serum glucose concentrations were analyzed with an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method (50) using the Dimension Xpand Clinical Chemistry System (Siemens Medical Diagnostics). Insulin was measured using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Siemens Immulite 1000; Siemens Healthcare Diagnostics Product Ltd.). Insulin intra-assay and interassay CVs were 5.7% and 6.7%, respectively. The HOMA-IR (51) was used to estimate insulin resistance. TNF-R2 concentrations were measured with the Ultra-Sensitive kit from Meso Scale Discovery using a MESO QuickPlex SQ 120. Plasma concentrations of leptin, oxidized LDL (oxLDL), and total adiponectin and high-molecular-weight (HMW) adiponectin were measured using ELISA kits from R&D Systems Inc., Mercodia AB, and Alpco, respectively. Serum concentrations of IL-6 were analyzed using an ELISA kit from R&D Systems Inc. and serum C-reactive protein (CRP) concentrations were measured by ELISA from Immundiagnostik AG.

### Clinical assessment and body composition

Blood pressure, temperature, and pulse were measured at each study visit after the participant had been seated and rested for  $\geq$ 5 min. Weight was measured to the nearest 0.1 kg using a digital scale at each study visit. Height was measured to the nearest 0.1 cm at week –4 and week 16 using a wall-mounted stadiometer.

DXA equipped with Corescan (Lunar iDXA; GE Healthcare) was used to determine whole-body composition and visceral adipose tissue at weeks 0 and 16. Data are reported as mass (kg) and mass normalized to BMI. To assess the validity of this instrument in the study population, 16 participants consented to have the scan twice at the week 0 visit. The CV was 0.37% for lean muscle mass (LMM), 1.09% for appendicular lean mass (ALM), 0.88% for trunk adipose, 0.42% for total adipose, and 5.27% for Corescan visceral fat.

### Diet assessment

Dietary intake was assessed in all participants between week –4 and week 0. Participants were instructed to keep one 3-d food record (1 weekend day and 2 weekdays). A registered dietitian reviewed and completed the food records with the participants. The data were entered into Nutrition Data Systems for Research (NDSR, version 2014, University of Minnesota). Dietary data, except kilocalories (kcal), are expressed per 1000 kcal consumed.

### Statistical analysis

All statistical analysis was performed with a significance level of 0.05. Analysis of oxylipins and other lipid mediators was performed using JMP Pro 14.1 (JMP, SAS Institute). Before analysis, outliers in the metabolite values were removed using the robust Huber M test. Next, data were transformed to obtain normal distributions using Johnson's transformation. Differences in metabolite mean concentrations were assessed using ANCOVA, with the time of visit as fixed effect and subject as covariate. This approach emphasized the treatment effect (difference between week 0 and week 16) and reduced interindividual variance. The *P*-value threshold was adjusted using Benjamini–

**TABLE 1** Participant characteristics<sup>1</sup>

Characteristic	n = 16
Age, y	59.8 ± 5.1
Cessation of menses, y	9.1 ± 8.8
Race	
Caucasian	81 (13)
Black or African American	19 (3)
Education	
High school diploma/some college	50 (8)
4-Y degree/some graduate school	19 (3)
Graduate degree	31 (5)
Use of lipid-lowering medications	6 (1)
Use of blood pressure-lowering medications	13 (2)
Dietary intake	
Energy, kcal/d	1663 ± 486
Protein, g/1000 kcal	46 ± 11
Carbohydrate, g/1000 kcal	115 ± 16
Total fat, g/1000 kcal	41 ± 5.1
Saturated fat, g/1000 kcal	14 ± 3.0
Monounsaturated fat, g/1000 kcal	14 ± 2.3
Polyunsaturated fat, g/1000 kcal	9.4 ± 2.7
Linoleic acid, g/1000 kcal	8.1 ± 2.3

<sup>1</sup>Values are mean ± SD or % (n) of participants.

Hochberg false discovery rate correction with a  $q = 0.2$  (52). The fold difference used in the oxylipin/endocannabinoid network visualizations was calculated by dividing the week-16 time point mean by the week-0 time point mean. Group means were calculated using normally distributed data.

For the remaining analyses, paired  $t$  tests were used to test for differences in measures from the prebaseline visit (week -4) to the baseline visit (week 0). For analysis of measurements during the intervention period (weeks 0-16), linear mixed models with random intercepts to account for within-subject correlation over time were fitted for each measure. All 16 participants who completed the baseline visit (week 0) were included in these models. If there were significant changes in an outcome over time (based on the test of the week effect), linear contrasts in the mixed models were used to test for differences from baseline (week 0) to each of weeks 4, 8, 12, and 16. All tables show means ± SDs. The ratio of adiponectin to leptin was log transformed for modeling to better approximate normality of residuals. Hypothesis tests were conducted at a 5% type 1 error rate. Adjustments for multiple comparisons were not made, because this is a hypothesis-generating pilot study with a limited sample size. However, only testing for differences from baseline in models with a significant week effect helped reduce the chance of a false finding (i.e., reduced type 1 error). Statistical analyses were conducted using Stata version 15 (53).

**TABLE 2** Adherence to OA-oil and LA-oil consumption<sup>1</sup>

	Week -4 to week 0	Week 0 to week 4	Week 4 to week 8	Week 8 to week 12	Week 12 to week 16
Weight, %	92 ± 38 (16)	81 ± 32 (16)	79 ± 33 (14)	78 ± 28 (14)	79 ± 23 (13)
Self-record, %	96 ± 5 (5)	96 ± 4 (9)	92 ± 8 (8)	93 ± 10 (6)	90 ± 11 (7)

<sup>1</sup>Values are mean ± SD (n); specifically, mean ± SD percentage of adherence (expected oil consumption) from either weight of the oil or self-record of daily teaspoon consumption. OA-oil consumption week -4 to week 0; LA-oil consumption week 0 to week 16. Participants were instructed to consume 9.3 g or 10 mL/d (2 teaspoons) of oil. LA-oil, linoleic acid-rich oil; OA-oil, oleic acid-rich oil.

## Results

### Participants' characteristics and adverse events

Eighteen participants enrolled and 15 completed the study. Two withdrew before the week 0 visit and 1 before the week 8 visit. Reasons for withdrawal included adverse events (related or unrelated) and scheduling conflicts. There were 11 reported adverse events related to the consumption of the study oils (5 with OA-oil and 6 with LA-oil). All adverse events were gastrointestinal complaints with 72% of them described as mild and 64% resolving before being reported.

**Table 1** reports characteristics of the participants who completed at least the week 0 study visit ( $n = 16$ ). The current DRI for linoleic acid is the Adequate Intake of 11 g/d for women aged  $\geq 51$  y (54). Dietary assessment revealed that the mean habitual intake of LA was  $13.7 \pm 6.2$  g/d and with the addition of the LA-oil the mean intake increased to  $20.6 \pm 6.2$  g/d.

### Adherence and changes in measurements during the run-in period

The mean consumption of the OA-oil during the 4-wk run-in period was 92% ( $n = 16$ ) of that expected from the oil weight and 96% ( $n = 5$ ) of that expected from self-report (**Table 2**). The OA-oil did not alter plasma or erythrocyte LA or OA concentrations (**Supplemental Table 2**). Fasting serum glucose ( $P = 0.0001$ ) and fasting plasma total adiponectin concentrations ( $P < 0.0001$ ) decreased whereas CRP ( $P = 0.046$ ) increased from week -4 to week 0. There were no other changes in any of the study measurements measured after the 4-wk run-in period.

### Adherence and changes in measurements during the intervention period

Participants were instructed to consume 10 mL (2 teaspoons) of the LA-oil per day during the 16-wk intervention period. Adherence measured by the oil weight was similar throughout the intervention period with a mean of 81% (weeks 0-4;  $n = 16$ ), 79% (weeks 4-8;  $n = 14$ ), 78% (weeks 8-12;  $n = 14$ ) and 79% (weeks 12-16;  $n = 13$ ). During the intervention period fewer participants completed the self-reported oil consumption at each visit than returned the unused oil. Self-reported adherence was on average 96% (weeks 0-4;  $n = 9$ ), 92% (weeks 4-8;  $n = 8$ ), 93% (weeks 8-12;  $n = 6$ ), and 90% (weeks 12-16;  $n = 7$ ).

**Table 3** presents plasma, erythrocyte, and adipose tissue fatty acid composition results. LA in plasma, erythrocytes, and adipose tissue did not change significantly between week 0 and week 16. However, in both plasma and erythrocytes LA significantly increased ( $P = 0.05$ ) at weeks 8 and 12 compared with week 0. AA was unchanged in erythrocytes ( $P = 0.86$ ) and adipose tissue ( $P = 0.30$ ), but decreased in plasma

**TABLE 3** Fatty acids composition during the LA-rich oil intervention period<sup>1</sup>

	Week 0	Week 4	Week 8	Week 12	Week 16	P value <sup>2</sup>
<b>Plasma</b>						
OA	19 ± 1.7	18 ± 2.7	18 ± 2.3	17 ± 1.9	18 ± 2.10	0.07
ALA	0.65 ± 0.21	0.64 ± 0.19	0.60 ± 0.17	0.55 ± 0.16	0.62 ± 0.17	0.13
LA	31 ± 4.8	32 ± 4.5	33 ± 4.1*	33 ± 4.3*	32 ± 4.3	0.05
AA	7.6 ± 2.5	7.3 ± 2.5	7.1 ± 2.1*	7.4 ± 2.1*	7.2 ± 2.4*	0.01
EPA	0.47 ± 0.13	0.53 ± 0.17	0.47 ± 0.14	0.49 ± 0.12	0.45 ± 0.09	0.32
DHA	2.3 ± 0.45	2.2 ± 0.67	2.0 ± 0.41	2.0 ± 0.40	2.1 ± 0.33	0.22
<b>Erythrocyte</b>						
OA	14 ± 1.1	13 ± 0.78	14 ± 1.0	13 ± 0.95	13 ± 1.2	0.49
ALA	0.17 ± 0.06	0.16 ± 0.07	0.14 ± 0.06	0.13 ± 0.06*	0.18 ± 0.06	0.01
LA	13 ± 2.1	14 ± 2.0	14 ± 1.9*	14 ± 2.1*	14 ± 1.6	0.05
AA	15 ± 2.1	16 ± 2.0	16 ± 2.0	16 ± 1.8	16 ± 1.6	0.86
EPA	2.1 ± 0.38	2.1 ± 0.36	2.1 ± 0.31	2.0 ± 0.33	2.0 ± 0.32	0.39
DHA	3.4 ± 0.89	3.3 ± 0.86	3.3 ± 0.77	3.1 ± 0.55	3.2 ± 0.68	0.32
<b>Adipose tissue</b>						
OA	44 ± 2.4	–	–	–	45 ± 2.9	0.16
ALA	1.0 ± 0.26	–	–	–	1.0 ± 0.25	0.60
LA	20 ± 2.8	–	–	–	20 ± 3.3	0.14
AA	0.59 ± 0.25	–	–	–	0.54 ± 0.22	0.30
EPA	0.21 ± 0.058	–	–	–	0.22 ± 0.084	0.62
DHA	0.16 ± 0.079	–	–	–	0.13 ± 0.066	0.07

<sup>1</sup>Values are mean ± SD percentages of total fatty acids. \*Significantly different from week 0 at  $P < 0.05$  level. Plasma and erythrocyte tests: week 0,  $n = 16$ ; week 4,  $n = 16$ ; week 8,  $n = 15$ ; week 12,  $n = 14$ ; week 16,  $n = 15$ . Adipose tests: week 0,  $n = 12$ ; week 16,  $n = 10$ . AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; LA, linoleic acid; OA, oleic acid.

<sup>2</sup>P value from visit effect in linear mixed model.

( $P = 0.01$ ) starting at week 8 and remained lower than week 0 at both weeks 12 and 16.  $\alpha$ -Linolenic acid (ALA; 18:3n-3) decreased at week 8 in erythrocytes but did not change in plasma ( $P = 0.13$ ) or adipose tissue ( $P = 0.60$ ). OA, EPA, and DHA (22:6n-3) were all unaltered in plasma, erythrocytes, and adipose tissue during LA-oil supplementation.

Total adiponectin increased at week 4 during the intervention period and remained higher at week 16 than at week 0 (Table 4). HMW adiponectin was also significantly increased from week 0 to week 16 of the intervention period ( $P < 0.0001$ ). No changes were seen in leptin,

TNF-R2, and CRP, but IL-6 concentrations were altered during the intervention period ( $P = 0.0007$ ), increasing from week 0 to week 8 and then decreasing from week 8 to week 16. As a result, only the week 8 concentration was significantly different from week 0. Glucose ( $P < 0.001$ ), insulin ( $P = 0.03$ ), and HOMA-IR values ( $P = 0.004$ ) transiently increased over the intervention period but were unchanged by week 16 compared with week 0. Starting at week 4, HOMA-IR and glucose were elevated (compared with week 0) and remained higher at weeks 8 and 12, whereas insulin concentrations rose at weeks 8 and 12. HDL, LDL,

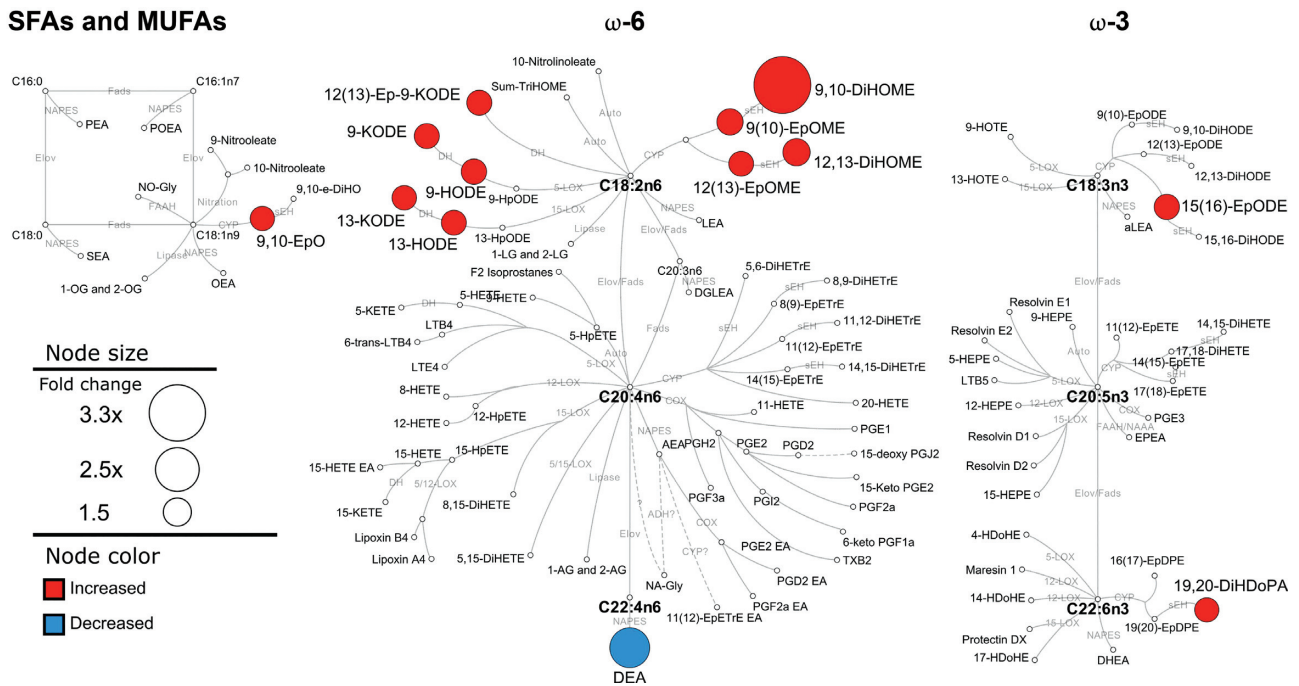
**TABLE 4** Biochemical measurements during the LA-oil intervention period<sup>1</sup>

	Week 0	Week 4	Week 8	Week 12	Week 16	P value <sup>2</sup>
Total adiponectin, $\mu\text{g/mL}$	6.8 ± 2.7	9.0 ± 3.2*	8.4 ± 3.0*	9.1 ± 3.5*	8.3 ± 3.2*	<0.0001
HMW adiponectin, $\mu\text{g/mL}$	3.7 ± 2.0	–	–	–	4.9 ± 2.1*	<0.0001
Leptin, ng/mL	30 ± 19	–	27 ± 21	–	29 ± 23	0.61
Adiponectin to leptin ratio <sup>3</sup>	0.3 ± 0.3	–	0.4 ± 0.2*	–	0.5 ± 0.6*	0.0058
IL-6, pg/mL	2.3 ± 1.6	2.7 ± 1.6	3.5 ± 2.3*	2.9 ± 1.3	1.9 ± 0.89	0.0007
oxLDL, U/L	72 ± 21	66 ± 17	64 ± 16	62 ± 16	64 ± 19	0.09
CRP, ng/mL	5445 ± 4676	3658 ± 3428	–	–	4079 ± 3653	0.09
TNF-R2, pg/mL	5074 ± 1346	5266 ± 1138	5313 ± 1209	5089 ± 1212	5055 ± 1162	0.24
Glucose, mg/dL	92 ± 9.5	101 ± 10*	100 ± 7.6*	100 ± 10*	95 ± 11	<0.0001
Insulin, $\mu\text{U/mL}$	12 ± 5.7	12 ± 4.8	13 ± 6.9*	13 ± 6.4*	11 ± 4.9	0.03
HOMA-IR	2.6 ± 1.3	3.2 ± 1.4*	3.1 ± 1.8*	3.3 ± 1.7*	2.6 ± 1.3	0.004
Triglycerides, mg/dL	146 ± 50	153 ± 81	152 ± 60	136 ± 46	150 ± 57	0.82
HDL cholesterol, mg/dL	54 ± 15	54 ± 15	55 ± 14	54 ± 15	55 ± 16	0.87
LDL cholesterol, mg/dL	112 ± 29	111 ± 36	107 ± 28	111 ± 27	109 ± 24	0.93
Total cholesterol, mg/dL	196 ± 35	196 ± 43	192 ± 35	193 ± 31	195 ± 29	0.92

<sup>1</sup>Values are means ± SDs. \*Significantly different from week 0 at  $P < 0.05$  level;  $n$  varies from 14 to 16. CRP, C-reactive protein; HMW, high molecular weight; LA-oil, linoleic acid-rich oil; oxLDL, oxidized low-density lipoprotein; TNF-R2, TNF-receptor 2.

<sup>2</sup>P value from visit effect in linear mixed model.

<sup>3</sup>Log transformed for analysis but shown as untransformed means ± SDs at each time point.



**FIGURE 1** Changes in oxylipins and acylethanolamides from week 0 to week 16 of LA-oil supplementation. Network presents fatty acid metabolic pathways, including SFAs and MUFAs and  $\omega$ -3 and  $\omega$ -6 fatty acids with oxylipins and endocannabinoid synthesis pathways. Node size represents the fold change and the color represents the directionality of the change: red, increased after 16 wk; blue, decreased after 16 wk. ADH, alcohol dehydrogenase; AEA, arachidonylethanolamine; AG, arachidonoylglycerol; COX, cyclooxygenase; CYP, cytochrome P450; DEA, docosatetraenylethanolamide; DGLEA, dihomo- $\gamma$ -linolenoyl ethanolamide; DH, dehydrogenase; DHEA, docosahexaenoyl ethanolamide; DiHDoPA, dihydroxydocosapentaenoic acid; DiHETrE, dihydroxyeicosatrienoic acid; DiHO, dihydroxyoctadecanoic acid; DiHODE, dihydroxyoctadecadienoic acid; DiHOME, dihydroxyoctadecenoic acid; EA, ethanolamide; Elov, fatty acids elongase; EpETrE, epoxyeicosatrienoic acid; EpO, epoxyoctadecanoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; FAAH, fatty acid amide hydrolase; Fads, fatty acid desaturase; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HOTE, hydroxyoctadecatrienoic acid; HpETE, hydroperoxyl-eicosatetraenoic acid; HpODE, hydroperoxyoctadecadienoic acid; KETE, keto-eicosatetraenoic acid; KODE, keto-octadecadienoic acid; LG, linoleoylglycerol; LOX, lipoxygenase; LTB, leukotriene B; LTE, leukotriene E; NAPES, N-acylphosphatidylethanolamine-specific; OEA, oleoylethanolamine; OG, oleoylglycerol; PEA, palmitoylethanolamine; PGD, prostaglandin D; PGE, prostaglandin E; PGF, prostaglandin F; PGI, prostaglandin I; PGJ, prostaglandin J; POEA, palmitoleoyl ethanolamide; SEA, stearoyl ethanolamide; sEH, soluble epoxide hydrolase; TriHOME, trihydroxyoctadecanoic acid.

and total cholesterol, oxLDL, and triglyceride concentrations were unaltered with LA-oil supplementation.

Concentrations of esterified oxylipins and acylethanolamides in the plasma were measured at week 0 and week 16 (Figure 1). Nine LA-oxylipins significantly increased from week 0 to week 16 (Supplemental Table 3). 9,10-dihydroxyoctadecenoic acid (DiHOME) had the largest increase followed by 12,13-DiHOME, 9(10)-epoxyoctadecenoic acid (EpOME), 12(13)-EpOME, 13-hydroxyoctadecadienoic acid (HODE), 13-keto-octadecadienoic acid (KODE), 9-HODE, 9-KODE, and 12(13)-Ep-9-KODE, all with a 1.5-fold increase. One OA-oxylipin, 9,10-epoxyoctadecanoic acid (EpO), 1 ALA-oxylipin, 15(16)-epoxyoctadecadienoic acid (EpODE), and 1 DHA-oxylipin, 12,20-dihydroxydocosapentaenoic acid (DiHDoPA), also increased from week 0 to week 16. The only acylethanolamide altered during the intervention period was the AA-derived docosatetraenylethanolamide (DEA), which decreased.

Body composition analyzed by DXA at week 0 and week 16 revealed no changes in total fat, visceral fat, trunk fat, or LMM/BMI in the women (Table 5). ALM/BMI increased by 3%, but it was not statistically significant ( $P = 0.06$ ). BMI, blood pressure, temperature, and pulse were unaltered during the intervention period (Table 6).

**TABLE 5** Body composition measurements during the LA-oil intervention period<sup>1</sup>

	Week 0	Week 16	$P$ value <sup>2</sup>
Total fat, kg	34 $\pm$ 8.4	34 $\pm$ 9.7	0.30
Visceral fat, kg	1.0 $\pm$ 0.39	1.0 $\pm$ 0.42	0.64
Trunk fat, kg	19 $\pm$ 4.5	18 $\pm$ 5.2	0.28
ALM/BMI	0.65 $\pm$ 0.064	0.67 $\pm$ 0.056	0.06
LMM/BMI	1.4 $\pm$ 0.15	1.4 $\pm$ 0.15	0.16

<sup>1</sup>Values are means  $\pm$  SDs. Week 0,  $n = 14$ ; week 16,  $n = 13$ . ALM, appendicular lean mass; LA-oil, linoleic acid-rich oil; LMM, lean muscle mass.

<sup>2</sup> $P$  value from visit effect in linear mixed model.

**TABLE 6** Vital signs and BMI during the LA-oil intervention period<sup>1</sup>

	Week 0	Week 4	Week 8	Week 12	Week 16	P value <sup>2</sup>
Pulse, bpm	70 ± 9.3	71 ± 9.0	75 ± 11	74 ± 9.4	71 ± 11	0.10
Temperature, °C	36 ± 0.43	36 ± 0.39	36 ± 0.43	36 ± 0.41	36 ± 0.30	0.16
Systolic BP, mm Hg	125 ± 16	124 ± 17	130 ± 15	129 ± 13	122 ± 15	0.14
Diastolic BP, mm Hg	79 ± 7.4	78 ± 7.8	80 ± 7.4	78 ± 7.4	78 ± 9.2	0.62
BMI	31 ± 4.3	31 ± 4.7	31 ± 4.8	31 ± 4.9	31 ± 5.1	0.94

<sup>1</sup>Values are means ± SDs. \*Significantly different from week 0 at  $P < 0.05$  level. Week 0,  $n = 16$ ; week 4,  $n = 16$ ; week 8,  $n = 15$ ; week 12,  $n = 15$ ; week 16,  $n = 15$ . BP, blood pressure; LA-oil, linoleic acid-rich oil.

<sup>2</sup>P value from visit effect in linear mixed model.

## Discussion

The risk of MetS increases in women after menopause (12) and MetS increases the likelihood of developing heart disease (5–7) and type 2 diabetes (7, 8). Despite studies showing LA can reduce inflammation and favorably influence glycemia and body composition in women with diabetes (28, 29) and healthy men and women (31), little is known about the effect of LA in MetS. Alarming, the supply of LA in the US diet is decreasing as more plant seed oil crops are of the high-OA variety (26, 27). The objectives of this single-arm open-labeled pilot study were to investigate the effects of consuming an oil rich in LA on adiponectin concentrations, fatty acid and oxylipin profiles, and parameters of MetS and to evaluate oil consumption adherence in postmenopausal women with MetS.

There was a transient increase of LA concentrations at weeks 8 and 12; however, the 3% and 4% increase in plasma and erythrocyte LA, respectively, was not significantly different between week 0 and week 16. This is in contrast to our previous study, where a similar amount of LA supplementation, provided to participants in capsule form, increased serum LA concentrations by 11% after 16 wk in a larger cohort of postmenopausal women with type 2 diabetes (28). However, unlike our previous study, the participants in this study were asked to measure out 10 mL/d of the provided study oil using a teaspoon (also provided). The delivery of loose oil may have increased the variability of the actual dose consumed.

Lower concentrations of adiponectin are associated with MetS (18), insulin resistance (55), and risk of type 2 diabetes (56). Both total adiponectin and HMW adiponectin were increased at week 16 compared with week 0, by 22% and 32%, respectively. Whereas the elevation of adiponectin was sustained during the 16-wk intervention period, there was a counterintuitive transient increase in glucose, insulin, and HOMA-IR, which returned to baseline levels at week 16. This is in contrast to our previous study where LA-oil supplementation increased adiponectin by 30% but decreased glucose and HOMA-IR in postmenopausal women with diabetes (28, 29). This lack of a consistent change in glycemic measurements may be linked to the less severe hyperglycemia in this cohort of women with MetS. Furthermore, it is possible that the adiponectin and the markers of glycemia during the intervention period were influenced by the initial decrease of adiponectin and glucose during the run-in period (week –4 to week 0). It is not clear if the increase in adiponectin and glucose during the intervention period would have occurred had there not also been a decrease from week –4 to week 0 when the women consumed the OA-oil. Further examination into effects of both LA and OA on adiponectin and glucose is

needed. A study of longer duration, adequate power, or more standardized dosing, e.g., delivery of oil by providing mixed foods or supplements, is needed to fully understand the effects of LA on these markers of glycemia in women with MetS.

Oxylipins have a variety of physiological functions (33, 34) and LA-oxylipins in particular may play a role in energy metabolism (57–59) and inflammation (60). 9,10-DiHOME and 12,13-DiHOME are inversely associated with BMI (57, 61) and waist circumference (61) in healthy individuals. 12,13-DiHOME is also negatively associated with HOMA-IR (57, 59), triglyceride concentrations (57), and adipose mass (59). In mice, 12,13-DiHOME can amplify fatty acid uptake in brown adipose tissue (57) and skeletal muscle (58). 13-HODE and 9-HODE can reduce adipocyte lipid storage and adipokine production in adipocytes (62) and may also play a role in atherosclerosis (63). Although concentrations of 9(10)-EpOME and 12(13)-EpOME are positively associated with microvascular function (64), women with type 2 diabetes have higher concentrations of these epoxides than women without diabetes (48). To our knowledge only 2 other studies have assessed the impact of increasing LA in the diet for 4 wk on oxylipin concentrations in humans (64–66). In the current study an analysis of changes in total esterified oxylipins after 16 wk of LA-oil supplementation was conducted. Not only were LA-oxylipins increased at week 16, but OA-, ALA-, and DHA-oxylipins were as well. The relative change in the 9 LA-oxylipins that increased was larger than the relative change of either plasma or erythrocyte LA. This is consistent with a previous study in men that saw larger relative increases in many LA-oxylipins than in plasma LA after 4 wk of safflower oil supplementation (66). In addition, this increase in LA-oxylipins, in spite of a lack of change in LA, was previously seen in the serum of rats fed a diet with safflower oil for 6 wk (67). In this study, 13-HODE and 9-HODE, both believed to be PPAR $\gamma$  agonists (37–39), demonstrated a 28% and 30% increase, respectively, which is similar to the percentage increases in total adiponectin and HMW adiponectin, suggesting a potential role of these oxylipins in altering adiponectin concentrations. However, more research is needed to identify the physiological effects of LA-oil supplementation, LA-oxylipins, and MetS.

There are several limitations to this study. First, this is a small, open-labeled pilot study in women only, with each individual acting as her own control, as opposed to an independent and double-blinded control group. Second, because there was no control group without LA-oil and there was no washout period between the run-in period using OA-oil and the commencement of using LA-oil at week 0, the results of the 16-wk intervention period may have been influenced by some carryover effects from the run-in period. Third, the daily oil dose was not premea-



sured for the study participants, increasing the likelihood of variation in the oil consumed per person each day. Fourth, 2 of the participants were given grapeseed oil for 4 or 8 wk, which, although it has a nearly identical fatty acid profile to the high-LA safflower oil, is not identical in terms of other compounds including polyphenols and antioxidants. Lastly, the generalizability of our findings to a broader group of people with MetS, e.g., men, youth, and elderly, is not realistic given the limited size of the cohort.

In conclusion, the increases of total and HMW adiponectin and LA-oxylipins after 16 wk of LA-oil supplementation merit further investigation. A future study should evaluate the effects of supplementation of LA in men and women with MetS to improve insulin sensitivity and affect cellular pathways downstream from adiponectin in a large, randomized, placebo-controlled clinical trial.

### Acknowledgments

The authors' responsibilities were as follows—RMC, MAB, and RA: wrote the paper; RMC, SP, J-YK, BO, and DB: conducted the research; MA-R, KH, RA, JWN, and KB: performed the statistical analysis; RA, JWN, and KB: analyzed the data; MAB: designed the research and had primary responsibility for the final content; and all authors: read and approved the final manuscript.

### References

- National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106(25):3143–421.
- Grundy SM, Brewer HB, Jr, Cleeman JI, Smith SC, Jr, Lenfant C; American Heart Association, National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109(3):433–8.
- Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. *Lancet* 2005;366(9491):1059–62.
- Chen S-J, Yen C-H, Huang Y-C, Lee B-J, Hsia S, Lin P-T. Relationships between inflammation, adiponectin, and oxidative stress in metabolic syndrome. *PLoS One* 2012;7(9):e45693.
- Ingelsson E, Sullivan LM, Murabito JM, Fox CS, Benjamin EJ, Polak JF, Meigs JB, Keyes MJ, O'Donnell CJ, Wang TJ, et al. Prevalence and prognostic impact of subclinical cardiovascular disease in individuals with the metabolic syndrome and diabetes. *Diabetes* 2007;56(6):1718–26.
- Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. The metabolic syndrome and cardiovascular risk: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;56(14):1113–32.
- Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735–52.
- Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. *Diabetes Care* 2008;31(9):1898–904.
- Moore JX, Chaudhary N, Akinjemiju T. Metabolic syndrome prevalence by race/ethnicity and sex in the United States, National Health and Nutrition Examination Survey, 1988–2012. *Prev Chronic Dis* 2017;14:E24.
- Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287(3):356–9.
- Park Y-W, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. *Arch Intern Med* 2003;163(4):427–36.
- Janssen I, Powell LH, Crawford S, Lasley B, Sutton-Tyrrell K. Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. *Arch Intern Med* 2008;168(14):1568–75.
- Morita Y, Iwamoto I, Mizuma N, Kuwahata T, Matsuo T, Yoshinaga M, Douchi T. Precedence of the shift of body-fat distribution over the change in body composition after menopause. *J Obstet Gynaecol Res* 2006;32(5):513–6.
- Bea JW, Thomson CA, Wertheim BC, Nicholas JS, Ernst KC, Hu C, Jackson RD, Cauley JA, Lewis CE, Caan B, et al. Risk of mortality according to body mass index and body composition among postmenopausal women. *Am J Epidemiol* 2015;182(7):585–96.
- Lovejoy JC, Champagne CM, de Jonge L, Xie H, Smith SR. Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int J Obes* 2008;32(6):949–58.
- Pu D, Tan R, Yu Q, Wu J. Metabolic syndrome in menopause and associated factors: a meta-analysis. *Climacteric* 2017;20(6):583–91.
- You T, Yang R, Lyles MF, Gong D, Nicklas BJ. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. *Am J Physiol Endocrinol Metab* 2005;288(4):E741–7.
- Yuan G, Qian W, Pan R, Jia J, Jiang D, Yang Q, Wang S, Liu Y, Yu S, Hu H, et al. Reduced circulating oxytocin and high-molecular-weight adiponectin are risk factors for metabolic syndrome. *Endocr J* 2016;63(7):655–62.
- Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, Shimomura I. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003;52(7):1655–63.
- Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995;270(45):26746–9.
- Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004;279(13):12152–62.
- Tamakoshi K, Yatsuya H, Wada K, Matsushita K, Otsuka R, Yang PO, Sugiura K, Hotta Y, Mitsuhashi H, Takefuji S, et al. The transition to menopause reinforces adiponectin production and its contribution to improvement of insulin-resistant state. *Clin Endocrinol (Oxf)* 2007;66(1):65–71.
- Milewicz A, Jedrzejuk D, Dunajska K, Lwow F. Waist circumference and serum adiponectin levels in obese and non-obese postmenopausal women. *Maturitas* 2010;65(3):272–5.
- Banh TH, Puchala SE, Cole RM, Andridge RR, Kiecolt-Glaser JK, Belury MA. Blood level of adiponectin is positively associated with lean mass in women without type 2 diabetes. *Menopause* 2019;26(11):1311–7.
- Chedraui P, Pérez-López FR, Escobar GS, Palla G, Montt-Guevara M, Cecchi E, Genazzani AR, Simoncini T, Research Group for the Omega Women's Health Project. Circulating leptin, resistin, adiponectin, visfatin, adipisin and ghrelin levels and insulin resistance in postmenopausal women with and without the metabolic syndrome. *Maturitas* 2014;79(1):86–90.
- Tarrago-Trani MT, Phillips KM, Lemar LE, Holden JM. New and existing oils and fats used in products with reduced *trans*-fatty acid content. *J Am Diet Assoc* 2006;106(6):867–80.
- Crawford AWW, Chunling W, Jenkins DJ, Lemke SL. Estimated effect on fatty acid intake of substituting a low-saturated, high-oleic, low-linolenic soybean oil for liquid oils. *Nutrition Today* 2011;46(4):189–96.
- Norris LE, Collene AL, Asp ML, Hsu JC, Liu L-F, Richardson JR, Li D, Bell D, Osei K, Jackson RD, et al. Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. *Am J Clin Nutr* 2009;90(3):468–76.

29. Asp ML, Collene AL, Norris LE, Cole RM, Stout MB, Tang S-Y, Hsu JC, Belury MA. Time-dependent effects of safflower oil to improve glycemia, inflammation and blood lipids in obese, post-menopausal women with type 2 diabetes: a randomized, double-masked, crossover study. *Clin Nutr* 2011;30(4):443–9.
30. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012;95(5):1003–12.
31. Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson HE, Larsson A, Johansson L, Ahlström H, Arner P, Dahlman I, et al. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* 2014;63(7):2356–68.
32. Wu JHY, Marklund M, Imamura F, Tintle N, Ardisson Korat AV, de Goede J, Zhou X, Yang W-S, de Oliveira Otto MC, Kröger J, et al. Omega-6 fatty acid biomarkers and incident type 2 diabetes: pooled analysis of individual-level data for 39 740 adults from 20 prospective cohort studies. *Lancet Diabetes Endocrinol* 2017;5(12):965–74.
33. Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv Nutr* 2015;6(5):513–40.
34. Barquissau V, Ghandour RA, Ailhaud G, Klingenspor M, Langin D, Amri EZ, Pisani DF. Control of adipogenesis by oxylipins, GPCRs and PPARs. *Biochimie* 2017;136:3–11.
35. Shearer GC, Borkowski K, Puumala SL, Harris WS, Pedersen TL, Newman JW. Abnormal lipoprotein oxylipins in metabolic syndrome and partial correction by omega-3 fatty acids. *Prostaglandins Leukotrienes Essent Fatty Acids* 2018;128:1–10.
36. Kliewer S, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$ . *Proc Natl Acad Sci U S A* 1997;94(9):4318–23.
37. Itoh T, Fairall L, Amin K, Inaba Y, Szanto A, Balint BL, Nagy L, Yamamoto K, Schwabe JW. Structural basis for the activation of PPAR $\gamma$  by oxidized fatty acids. *Nat Struct Mol Biol* 2008;15(9):924–31.
38. Bull A, Steffensen KR, Leers J, Rafter JJ. Activation of PPAR  $\gamma$  in colon tumor cell lines by oxidized metabolites of linoleic acid, endogenous ligands for PPAR  $\gamma$ . *Carcinogenesis* 2003;24(11):1717–22.
39. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR $\gamma$ . *Cell* 1998;93(2):229–40.
40. Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. *Am J Clin Nutr* 1985;42(2):317–22.
41. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226(1):497–509.
42. Stoffel W, Chu F, Ahrens EH. Analysis of long-chain fatty acids by gas-liquid chromatography. *Anal Chem* 1959;31(2):307–8.
43. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride—methanol. *J Lipid Res* 1964;5:600–8.
44. Harris WS, Lemke SL, Hansen SN, Goldstein DA, DiRienzo MA, Su H, Nemeth MA, Taylor ML, Ahmed G, George C. Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* 2008;43(9):805–11.
45. Pottala JV, Espeland MA, Polreis J, Robinson J, Harris WS. Correcting the effects of  $-20^{\circ}\text{C}$  storage and aliquot size on erythrocyte fatty acid content in the Women's Health Initiative. *Lipids* 2012;47(9):835–46.
46. Belury MA, Kempa-Steczko A. Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* 1997;32(2):199–204.
47. Belury MA, Cole RM, Bailey BE, Ke J-Y, Andridge RR, Kiecolt-Glaser JK. Erythrocyte linoleic acid, but not oleic acid, is associated with improvements in body composition in men and women. *Mol Nutr Food Res* 2016;60(5):1206–12.
48. Grapov D, Adams SH, Pedersen TL, Garvey WT, Newman JW. Type 2 diabetes associated changes in the plasma non-esterified fatty acids, oxylipins and endocannabinoids. *PLoS One* 2012;7(11):e48852.
49. Agrawal K, Hassoun LA, Foolad N, Pedersen TL, Sivamani RK, Newman JW. Sweat lipid mediator profiling: a noninvasive approach for cutaneous research. *J Lipid Res* 2017;58(1):188–95.
50. Kunst A, Drager B, Ziegenhorn J. UV methods with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer H, editor. *Methods of enzymatic analysis*. VI. 3rd ed. Weinham (Germany): Verlag Chemie GmbH; 1983. pp. 163–72.
51. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412–9.
52. Benjamini Y, Yekutieli D. Quantitative trait loci analysis using the false discovery rate. *Genetics* 2005;171(2):783–9.
53. StataCorp. *Stata Statistical Software: Release 15*. College Station (TX): StataCorp LLC; 2017.
54. US Department of Health and Human Services (DHHS) and USDA. *2015–2020 Dietary Guidelines for Americans*. 8th ed. Washington (DC): US DHHS and USDA; 2015.
55. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- $\alpha$  expression. *Diabetes* 2003;52(7):1779–85.
56. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 2009;302(2):179–88.
57. Lynes MD, Leiria LO, Lundh M, Bartelt A, Shamsi F, Huang TL, Takahashi H, Hirshman MF, Schlein C, Lee A, et al. The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. *Nat Med* 2017;23(5):631–7.
58. Stanford KI, Lynes MD, Takahashi H, Baer LA, Arts PJ, May FJ, Lehnig AC, Middelbeek RJW, Richard JJ, So K, et al. 12,13-diHOME: an exercise-induced lipokine that increases skeletal muscle fatty acid uptake. *Cell Metab* 2018;27(5):1111–20.
59. Vasan SK, Noordam R, Gowri MS, Neville MJ, Karpe F, Christodoulides C. The proposed systemic thermogenic metabolites succinate and 12,13-diHOME are inversely associated with adiposity and related metabolic traits: evidence from a large human cross-sectional study. *Diabetologia* 2019;62(11):2079–87.
60. Vangaveti VN, Jansen H, Kennedy RL, Malabu UH. Hydroxyoctadecadienoic acids: oxidised derivatives of linoleic acid and their role in inflammation associated with metabolic syndrome and cancer. *Eur J Pharmacol* 2016;785:70–6.
61. Pickens CA, Sordillo LM, Zhang C, Fenton JI. Obesity is positively associated with arachidonic acid-derived 5- and 11-hydroxyeicosatetraenoic acid (HETE). *Metabolism* 2017;70:177–91.
62. Zahradka P, Neumann S, Aukema HM, Taylor CG. Adipocyte lipid storage and adipokine production are modulated by lipoxygenase-derived oxylipins generated from 18-carbon fatty acids. *Int J Biochem Cell Biol* 2017;88:23–30.
63. Vangaveti V, Baune BT, Kennedy RL. Hydroxyoctadecadienoic acids: novel regulators of macrophage differentiation and atherogenesis. *Ther Adv Endocrinol Metab* 2010;1(2):51–60.
64. Holt RR, Yim SJ, Shearer GC, Hackman RM, Djurica D, Newman JW, Shindel AW, Keen CL. Effects of short-term walnut consumption on human microvascular function and its relationship to plasma epoxide content. *J Nutr Biochem* 2015;26(12):1458–66.
65. Borkowski K, Yim SJ, Holt RR, Hackman RM, Keen CL, Newman JW, Shearer GC. Walnuts change lipoprotein composition suppressing TNF $\alpha$ -stimulated cytokine production by diabetic adipocyte. *J Nutr Biochem* 2019;68:51–8.
66. Lankinen MA, Fauland A, Shimizu B-i, Ågren J, Wheelock CE, Laakso M, Schwab U, Pihlajamäki J. Inflammatory response to dietary linoleic acid depends on *FADS1* genotype. *Am J Clin Nutr* 2019;109(1):165–75.
67. Leng S, Winter T, Aukema HM. Dietary LA and sex effects on oxylipin profiles in rat kidney, liver, and serum differ from their effects on PUFAs. *J Lipid Res* 2017;58(8):1702–12.